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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/886,044	06/30/97	BHATTACHARJEE A	71007/137

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EXAMINER	
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ART UNIT	**PAPER NUMBER**
1641	20

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Please find below and/or attached an Office communication concerning this application or proceeding.

Commissioner of Patents and Trademarks

Office Action Summary

Application No.	Applicant(s)
08/886,044	Bhattacharjee et al.
Examiner	**Group Art Unit**
S. Devi, Ph.D.	1641

☒ Responsive to communication(s) filed on *Jan 14, 1999*.

☒ This action is **FINAL**.

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *three* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *1-3, 5-8, 12-17, 19, and 20* is/~~are~~ pending in the application.

Of the above, claim(s) *12-14* is/~~are~~ withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *1-3, 5-8, 15-17, 19, and 20* is/~~are~~ rejected.

☐ Claim(s) _____ is/are objected to.

☒ Claims *1-3, 5-8, 12-17, 19, and 20* are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☐ Information Disclosure Statement(s), PTO-1449, Paper No(s). _____

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Amendment

1) Acknowledgment is made of Applicants' amendment filed 14 January 1999 (paper no. 27) in response to the non-final Office Action mailed 14 September 1998 (paper no. 23).

Claims Status

2) Claim 12 has been amended via paper no. 27.

New claims 19 and 20 have been added via paper no. 27.

Claims 1-3, 5-8, 12-17, 19 and 20 are pending. Claims 12-14 were withdrawn from further consideration.

Claims 1-3, 5-8, 15-17, 19 and 20 are under examination.

Prior Citation of Title 35 Sections

3) The text of those sections of Title 35 U.S. Code not included in this action can be found in a prior Office Action.

Prior Citation of References

4) The references cited or used as prior art in support of one or more rejections in the instant Office Action and not included on an attached form PTO-892 or form PTO-1449 have been previously cited and made of record.

Co-pending Application

5) It has come to the attention of the Examiner of record that Applicants have a co-pending application filed in the Office after the filing date of the instant application on 20 June 1997. The co-pending application, SN 08/467,047, was filed 6 June 1995 and has similar/identical claim(s) pending. In light of this finding, a double patenting rejection is made in this Office Action.

Rejection(s) Maintained

6) The rejection of claims 1-3, 5-8 and 15-17 under 35 U.S.C. § 103(a) as being unpatentable over Zollinger *et al.* (US 4,707,543) in view of Zeigler *et al.* (*New Eng. J. Med.* 307: 1225-1230, 1982) or Myers *et al.* (US 4,912,094) and Munford *et al.* (US 4,929,604) is maintained for reasons set forth therein and those that are set forth below.

Applicants' Arguments & the Office's Response

Applicants contend that:

(a) The teachings in Myers and Munford, taken in light of the art as a whole, would not have suggested that J5 LPS could form the basis for an effective active vaccine against sepsis.

The reference of Myers is cited in a 103 rejection to document the highly conserved nature of the core region "among LPSs obtained from different genera of *Enterobacteriaceae*" is known in the art, and that "immunity against the core region is ..... protective against a wide variety of Gram negative bacterial challenges" as "demonstrated by the work of Ziegler *et al.*" (see column 2, lines 9-13), and that "LPS prepared from ..... a strain that has a partially-complete (and therefore antigenically cross-reactive) core-region (e.g. ***E. coli* J5**)" can be "administered" (emphasis added) (column 10, lines 4-9).

The reference of Munford is used in a 103 rejection to document that "the structure of the lipid A moiety is highly conserved" in the LPS of many pathogenic bacteria including *Salmonella, Escherichia, Haemophilus* and *Neisseria*, and that LPSs may be used as vaccines to prevent gram negative bacterial sepsis by producing antibodies to R-core regions (see the abstract and column , lines 41-45). Munford also teaches that the structure of the R core region of LPS "is similar in most gram negative bacteria" (see column 1, lines 34-36).

Given the state of the art, the rejection stands even without the teachings of Myers *et al.* and Munford *et al.*

(b) The "art as whole contravenes any argument that there is a single highly-conserved core region, and would not have suggested that J5 LPS could form the basis for an effective vaccine against sepsis" and it is "applicants' combination of detoxified J5 LPS and OMP that presents the LPS to the immune system in an efficacious manner".

Contrary to Applicants' contention, the state of the art shows that J5 LPS has formed the basis for an active vaccine against sepsis. See Dunn *et al.* and Moore *et al.* below.

- Moore *et al.* (*Transplantation* 44: 249-253, 1987) teach active immunization of a mammalian subject against bacterial endotoxin with pure *E. coli* J5 LPS and passive immunization with an anti-J5 antiserum (see abstract and page 250). Active immunization with purified J5 LPS alone, without complexation or conjugation, produced a significant increase in antibody level (see page 251). See also under 'State of the Art'.

Dunn *et al.* (*Surgery* 96: 440-446, 1984) used purified J5 LPS (and J5 cells) as an immunogen in a mammal to raise antibodies that are cross-reactive *in vitro* and cross-protective *in vivo* against sepsis due to heterologous Gram negative bacteria including *Klebsiella* and *Pseudomonas*. Dunn *et al.* "*sought to test the ability of equine antibody directed against core endotoxin, a portion of bacterial outer membrane lipopolysaccharide common to many gram-negative microorganisms, to bind to various gram.-negative bacteria in vitro, to promote bacterial phagocytosis by leukocytes, and to protect against lethal gram-negative bacteremia in mice*" (Emphasis in original). As described above, Dunn *et al.* used purified J5 LPS (and J5 cells) as an immunogen in a mammal. Dunn *et al.* further teach that (see abstract):

> *Preimmunization IgG and F(ab')2 possessed no titer as determined by enzyme-linked immunosorbent assay, did not promote in vitro phagocytosis, and did not protect in vivo. Postimmunization IgG and F(ab')2 possessed a significant titer to E. coli J5 whole cell and lipopolysaccharide antigens and provided significant ($p < 0.05$) protection in vivo during lethal intravenous sepsis caused by either E. coli J5, E. coli O111:B4, Klebsiella pneumoniae, or Pseudomonas aeruginosa.*

The postimmunization IgG promoted *in vitro* phagocytosis of *E. coli* J5, *E. coli* O111:B4, *Klebsiella pneumoniae,* or *Pseudomonas aeruginosa*. Dunn *et al.* state (see pages 443 and 444):

> In the present study we demonstrated that immunization with *E. coli* J5 led to the development of immune antibody, which reacted primarily to the immunizing strain of bacteria but also extensively cross-reacted to a variety of serotypically distinct gram-negative microorganisms and types of LPS. Similar cross-reactive enhancement of phagocytosis was noted when immune IgG was compared with preimmunization IgG. ..... Pretreatment with either immune IgG or F(ab')2 before induction of sepsis conferred cross protection to four challenge organisms, three that were serotypically distinct from *E. coli* J5.
>
> Our results thus demonstrated that purified IgG directed against *E. coli* J5 whole cell and LPS antigens was cross-reactive in vitro and cross protective in vivo.

The J5 LPS-containing compositions and antisera raised with such compositions are known in the art to be cross-reactive with and cross-protective against heterologous Gram negative bacterial pathogens including *K. pneumoniae* and *P. aeruginosa*.

- For instance, Braude *et al.* (*J. Infect. Dis.* 136: Suppl. S167-S173, 1977) teach (see page S172):

> Whatever the mechanism of protection, it is clear that the J5 mutant of *E. coli* induces active and passive protection against overwhelming bacteremia due to a variety of gram-negative organisms ranging from different serotypes of *E. coli* to *K. pneumoniae* and *P. aeruginosa*. The J5 antiserum can be used successfully for treating overwhelming bacteremia in the complete absence of circulating PMLN and prevents death from bacteria that are resistant both to multiple antibiotics and to killing by serum and complement. Since human subjects can be safely immunized with the J5 vaccine, the development of immunotherapy against severe gram-negative bacterial infections warrants consideration.

> ...the same antiserum protects against both endotoxin and living bacteria.....

Braude *et al.* teach the opsonic and antitoxic properties of the antiserum against J5 in the statement:

> The opsonic activity of antibody to J5 is evident from its opsonic action against *P. aeruginosa* and by the accelerated clearance of *E. coli* O:4 from the circulation after treatment of agranulocytic rabbits with antiserum to J5 [9]. In the absence of polymorphonuclear leukocytes (PMLN), opsonization would still promote phagocytosis by the RES.

- See also Ziegler *et al.* (*J. Immunology* 111: 433-438, 1973) below.

(c) Studies of Lugowski *et al.* in which core LPS from *E. coli* was used as a vaccine, revealed no binding to *Klebsiella*, whereas "applicants' J5 LPS/OMP vaccine does bind to *Klebsiella*". The antibody taught by Di Padova (uncited art) binds to five known cores of *E. coli* and *Salmonella* core, but there was no binding to *Klebsiella* and *Ps. aeruginosa* whereas "applicants' J5 LPS/OMP vaccine does bind to *Ps. aeruginosa*".

First, the references of Lugowski *et al.* and Di Padova *et al.* were not cited as prior art by the Office. Secondly, the significance of Applicants' J5 LPS/OMP **vaccine** (as opposed antibodies elicited by the vaccine) **binding** to *Klebsiella* or *Ps. aeruginosa* is not understood. Further, the instant claims do not include the binding to *Klebsiella* as a limitation to distinguish their product or invention from those of the prior art. Even if this limitation was recited, the state of the art teaches that immunization of a mammal with purified *E. coli* J5 LPS (and *E. coli* J5 cells) induces antisera containing IgG antibodies that bind to the LPS of, and provide a significant protection against sepsis caused by, *E. coli* J5, *E. coli* K111:B4 (i.e., a heterologous *E. coli*), *Klebsiella pneumoniae,* and *Pseudomonas aeruginosa.* See Dunn *et al.* above.

(d) While there are highly conserved epitopes in the LPS core, a microheterogeneity exists in the epitopes and even within *E. coli*, there are significant differences between core epitopes.

The state of the art shows that despite the microheterogeneity occasionally reported by some workers, antibodies specific to the highly conserved epitope(s) in the J5 LPS core have been produced that are reactive with and protective against multiple heterologous Gram negative bacteria including *Klebsiella*, *Pseudomonas* sp. and multiple *E. coli* types. See Dunn *et al.*, and

Tomita under "State of the Art".

(e) A Medline search by one of the inventors revealed "[n]o disclosures of a J5 LPS/OMP vaccine other than applicants'". "All of the studies" listed in a review article by Cross *et al.* "used whole, killed bacterial preparations. **None used purified LPS** either alone or formulated with another component" (Emphasis added). "Indeed, the present inventors have found that detoxified LPS, given alone or as a conjugate, does not induce broadly cross-reactive antibodies" (see page 4 of Applicants' response).

The references that were obtained in a search done at the Office using Medline and other databases are discussed in this part of the Office Action. Applicants' attention is also drawn to the section created below that summarizes the 'State of the Art' at the time of the instant invention. See for example, Dunn *et al.* who used purified J5 LPS (and J5 cells) as an immunogen in a mammal to raise antibodies that are cross-reactive *in vitro* and cross-protective *in vivo* against sepsis due to heterologous Gram negative bacteria including *Klebsiella* and *Pseudomonas*. Moore *et al.* have produced a significant increase in antibody level following active immunization with purified J5 LPS alone, without complexation or conjugation (see page 251). See also Moore *et al.* under 'State of the Art'.

Contrary to Applicants' assertion, "purified" and "detoxified" J5 LPS has been "formulated with another component" and used as a vaccine which successfully induced antibodies cross-reactive with different heterologous Gram negative bacterial pathogens including *Klebsiella pneumoniae*. See Tomita (1994). Thus, detoxifying the purified J5 LPS (which is known to lack O-specific side chains and contain epitopes cross-reactive with heterologous Gram negative bacterial pathogens including *Klebsiella*) and "formulating it with another component" is not novel. Tomita also shows that the process of detoxification of the purified *E. coli* J5 LPS followed by formulation with a protein does not adversely affect the cross-reactive epitopes or the immunogenicity of the J5 LPS.

(f) The "evidence of unobviousness is found in an NIH review of a grant proposal by Dr. Cross to study J5 LPS/OMP vaccine". "The comments of the NIH reviewers are opinions rendered by independent experts in the field of sepsis prevention, regarding the novelty and inventiveness of applicants' claimed vaccine".

The NIH's review of a grant proposal is not based on M.P.E.P. or 35 Title Sections. The standards used by the NIH reviewers are not the same as those used by the Office.

(g) "Nothing in Zollinger would have motivated a skilled artisan to use J5 LPS in combination with an outer membrane protein, in place of the LPS disclosed in Zollinger". Applicants admit that Zollinger describes detoxified lipopolysaccharide-outer membrane complexes, but argue that the complexes were not tested for bactericidal antibody response (see page of Applicants' response).

The reference of Zollinger *et al.* is cited as the primary reference in a **103** rejection. Further, the instant claims do not recite "bactericidal antibody response" as a limitation. The state of the art teaches that J5 LPS induces protective antibodies, and Zollinger *et al.* teach that the disclosed complexes "induce immune response to bacterial infections" and "have activity against bacterial infections caused by gram negative bacteria including ....*Escherichia coli*..." (see column 12, lines 19-24).

(h) The purpose of the polysaccharide in Zollinger's teachings is to solubilize the outer membrane proteins. Replacing Zollinger's LPS with J5 LPS would lead to the conclusion that J5 LPS "would be expected to behave equivalently in combination with outer membrane protein in terms of the ability to solubilize outer membrane protein, since that is the purpose of the LPS in Zollinger".

There is no evidence that the J5 LPS in Applicants' composition or vaccine did not serve as a solubilizing agent since Applicants also used group B meningococcal OMP similar to Zollinger *et al.* Zollinger's detoxified LPS, irrespective of whether or not it was used as a solubilizing agent, on complexing with the group B meningococcal OMP yielded the "detoxified polysaccharide-outer membrane protein complexes" for use as "antibacterial vaccines" (see the title).

(I) Applicants question: "Would LPS without O-chains have been expected to solubilize outer membrane protein as effectively as LPS with O-chains?". Applicants go on to state: "Zollinger did not use LPS without side chains, and so provides no direct guidance on this issues.....A skilled artisan might doubt, as well, the ability of LPS without the O-chains effectively to solubilize outer membrane protein, thereby undermining the alleged case of

obviousness".

There appears to be no need for one to wonder or doubt as to whether an LPS without O-chains would solubilize the OMP as effectively as LPS with O-chains, because it is known to those skilled in the art that the LPS used in Zollinger's LPS-OMP complex **is** devoid of O-chains. The LPS of meningococcus (for example, used in Example 3) and the LPSs of other Gram negative bacterial pathogens taught by Zollinger *et al.*, for example, *Neisseria gonorrhoeae* and *Haemophilus influenzae* (see column 12, lines 22-24), do lack O-side chain(s). See Campnagari *et al.* (*Microb. Pathogen.* 8: 353-362, 1990) who explicitly teach that *N. meningitidis*, *N. gonorrhoeae* and *H. influenzae* "do not have repeating O-antigens as part of their principle surface glycolipid, the lipooligosaccharide (LOS)" (see abstract). Thus, Zollinger *et al.* provide "direct guidance" on the issue.

(j) There is suggestion of "uncertainty" in Zollinger's teachings over whether an LPS molecule modified to remove lipid A would retain the necessary solubilizing properties.

As noted by Applicants, Zollinger *et al.* teach that "the detoxified product was shown to **retain** its ability to bind to and solubilize outer membrane proteins" (see column 8, lines 66-68) (Emphasis added). Further, Tomita shows that the process of detoxification of J5 LPS followed by formulation with another protein does not adversely affect the cross-reactive epitopes or the immunogenicity of the J5 LPS. See also Seid *et al.* below under the section 'State of the Art'.

It is important note that Applicants detoxify their LPS by treating and heating in an alkaline solution followed by neutralization and recovery (see page 5 of the specification), similar to the teachings of Zollinger *et al.* (see Example 3).

(k) "Zollinger is not concerned with prevention or treatment of sepsis caused by multiple gram negative bacterial pathogens" (see page 10 of Applicants' response). "The purpose of the Zollinger composition is not the prevention or treatment of sepsis, let alone "sepsis caused by multiple gram negative bacterial pathogens" and LPS in Zollinger is not used "as an immunogen". "Zollinger is not concerned with making antibodies that provide broad-based protection against LPS endotoxin-mediated pathology".

Applicants are reminded that the reference of Zollinger *et al.* is cited in a **103** rejection.

Zollinger *et al.* teach that the detoxified polysaccharide-outer membrane protein complexes of their invention induces "immune response to bacterial infections". Zollinger *et al.* state that, more specifically, "evidence indicates that these complexes have activity against bacterial infections caused by gram-negative bacteria" including for example, *E. coli, Pseudomonas aeruginosa, Neisseria meningitidis*. It is reasonable to expect that Zollinger's detoxified *E. coli* LPS-OMP vaccine when modified with Ziegler's cross-reactive and cross-protective J5 LPS would serve as an effective vaccine that can be used for active or passive immunization of subjects against "infection by heterologous Gram-negative bacteria" as recited in instant claims. Instant claims do not recite phrases such as "sepsis caused by multiple gram negative bacterial pathogens". Even if such a limitation was recited, it is known to those skilled in the art that the "bacterial infections caused by gram-negative bacteria" such as *E. coli, Pseudomonas aeruginosa, Neisseria meningitidis* include sepsis.

Studies with Zollinger's vaccine have shown that the polysaccharide or LPS in the vaccine did act as an immunogen and induced polysaccharide- or LPS-specific antibodies (see Zollinger *et al., J. Clin. Invest.* 63: 836-848, 1979, and Zollinger *et al., Lancet* ii: 166, 1984).

(l) Ziegler *et al.* were "unable to identify antibodies as basis" for the protection induced by *E. coli* vaccine. Applicants discuss, at length, publications by others that were not cited in the Office's rejection which criticize Ziegler's work or study design.

To reiterate the teachings of Ziegler *et al.* as applied to the rejection of instant claims, Ziegler *et al.* teach a purified LPS of *E. coli* J5 and its role as an effective immunogen. Ziegler *et al.* teach that the "LPS of *E. coli* J5 lacks oligosaccharide side chains and that its core which is exposed is "nearly identical to that of most other gram-negative bacteria" (see the abstract). The J5 LPS-induced **antibodies** "**conferred** protection against Shwartzman reactions caused by purified endotoxins from bacterial species as widely varied as *E. coli*, *Salmonella typhimurium*, and the meningococcus", i.e. heterologous gram negative bacteria recited in claim 1 (see page 1226). Zeigler *et al.* describe the advantage of using *E. coli* J5 over that of its parent strain by stating that in case of LPS obtained from the parent *E. coli* strain, the "core determinants are concealed by side chains" (i.e. O-specific side chains). Zeigler *et al.* further teach the ineffectiveness of antiserum raised to the LPS of J5's parent *E. coli* (which has O-specific side

chain attached to the core determinant) against gram negative bacterial infections and also the **association** between high titers of antibodies to LPS-core determinants and low rates of shock and death observed in animal models. It is taught that this protection is independent of antibody to the O-specific side chain of LPS (see page 1226). Thus, Zeigler *et al.* clearly provide the motivation for one skilled in the art to use preferably *E. coli* J5 LPS that is devoid of O-specific side chain over it's parent *E. coli* strain, that has O-specific side chain intact, as an immunogen to treat sepsis caused by multiple gram negative bacterial pathogens. *E. coli.* Clearly, Ziegler *et al.* teach antibodies to J5 LPS as the basis for the protection illustrated.

(m) "The teaching of Ziegler herself....is that it likely was factors in the antisera other than LPS that were responsible for the protection observed" and therefore, a skilled artisan would not use a complex of J5 LPS and OMP to generate antibodies effective against LPS endotoxin-mediated pathology.

See above under paragraph (l) for why one skilled in the art would be motivated to use Ziegler's J5 LPS in place of Zollinger's generic *E. coli* LPS in his LPS-OMP complex.

Applicants' attention is also drawn to the teachings of Ziegler *et al.* (*J. Immunology* 111: 433-438, 1973) who disclose that the "treatment of agranulocytic animals with antiserum against the J5 mutant of *E. coli* O111 lowered the death rate from bacteremia due to the unrelated serotypes of *E. coli* O4 and O17 as well as the heterologous species, *K. pneumoniae*." (see page 436). The non-immune serum did not offer protection against *E. coli* O4 and O17, and *K. pneumoniae* (see Table I). Ziegler *et al.* state (see page 436):

> In contrast to the successful use of J5 antiserum in *K. pneumoniae* septicemia, *E. coli* O111 antiserum was **not** protective against Klebsiella. (Emphasis added).

These teachings of Ziegler *et al.* show that antibodies to "factors other than the LPS" in *E. coli* J5 did not provide protection against heterologous bacteria.

(n) Applicants submit a further declaration from Dr. Cross which provides results of challenge studies with heterologous bacteria. While the information in the declaration supports that provided in the specification, it does not overcome the rejection of instant claims under 35 U.S.C 103(a).

Summary

In summary, Applicants appear to argue that the combination of references fails because the prior art does not have anticipatory references regarding all elements of the invention. The argument is not persuasive. At issue is whether the claimed immunogenic composition, the vaccine and the methods are obvious over the prior-art composition, vaccine and methods, given the teachings of Zollinger *et al.* and Ziegler *et al.*, with or without Munford *et al.* or Meyer *et al.* It can hardly be argued that replacement of Zollinger's generic *E. coli* LOS with an alternative, functionally equivalent, detoxified J5 LOS core, similarly lacking O-specific side chain and additionally possessing cross-reactive/cross-protective epitopes, such as the one taught by Zielger *et al.* (or Tomita) would not be obvious to a skilled artisan, and when replaced, would not result in an effective vaccine capable of inducing antibodies cross-reactive/cross-protective against multiple heterologous Gram negative bacterial pathogens including *Klebsiella* and *Pseudomonas* sp. The invention as a whole, would have been obvious to a practitioner in view of the contemporary knowledge in the art at the time of invention and the state of the art at the time of the invention (see below) and the combined teachings of Zollinger *et al.* and Ziegler *et al.* It should be noted that what would reasonably have been known and used by one of ordinary skill in the art need not be explicitly taught. See *In re Nilssen*, 851 F.2d 1401, 7 USPQ2d 1500 (Fed. Cir. 1988). The test of obviousness is not express suggestion of the claimed invention in any and all of the references, but rather what the references taken collectively would reasonably have suggested to those of ordinary skill in the art presumed to be familiar with them. *In re Keller*, 642 F.2d 413, 425, 208 USPQ 871, 881 (CCPA 1981). Obviousness does not require absolute predictability, (see *In re Lamberti*, 192 USPQ 278), but only a reasonable expectation of success (see *In re O'Farrell*, 7 USPQ 2d 1673, Fed. Cir. 1988).

Zollinger *et al.*, the primary reference cited in the **103** rejection, discloses that combining or complexing of group B meningococcal OMP with detoxified LPS (lacking O-specific side chains) results in a vaccine, which when presented to a host's immune system serves as an efficacious immunogen. The elements of the instant claims, drawn to an immunogenic composition, a vaccine and method of active immunization, are taught by Zollinger *et al.* Zollinger *et al.* further teach similar complexes derived from multiple Gram negative bacteria including *Escherichia coli*. The various bacterial pathogens against which Zollinger states that his

vaccine complex is applicable, include *Haemophilus*, *Neisseria meningitidis*, and *Neisseria gonorrhoeae,* and it is known in the art that their LPSs lack O-specific side chains (Campagnari *et al.* (*Microb. Pathogen.* 8: 353-362, 1990). The *E. coli* LPS in the detoxified LPS-OMP complex taught by Zollinger is inclusive of J5 LPS. Zollinger, however, does not expressly teach his *E. coli* LPS to be J5 LPS.

However, Zeigler *et al.* clearly provide the motivation for one skilled in the art to preferably use *E. coli* J5 LPS lacking the O-specific side chain over it's parent *E. coli* strain by teaching that the "core determinants are concealed by side chains" (i.e. O-specific side chains) in the LPS obtained from the parent *E. coli* strain, and by teaching the ineffectiveness of antiserum raised to the LPS of J5's parent *E. coli* (which has O-specific side chain attached to the core determinant) against gram negative bacterial infections and by teaching the association between high titers of antibodies to LPS-core determinants and low rates of shock and death observed in animal models. Given the teachings of Zollinger *et al.* that a detoxified LPS from a Gram negative bacterial pathogen lacking O-specific side chain(s) in the LPS serves as an efficacious antibacterial vaccine on complexing with group B meningococcal OMP, it would have been obvious and reasonable for a skilled artisan to expect that an alternative detoxified Gram negative bacterial LPS, similarly lacking O-specific side chain(s) and containing a common epitope cross-reactive with and crossprotective against heterologous Gram negative bacterial pathogens including *Klebsiella* and *Pseudomonas*, when used in place of Zollinger's detoxified LPS in combination with group B meningococcal OMP, would, like wise, result in an efficacious vaccine and would further confer cross-protection against multiple heterologous Gram negative bacterial pathogens on active or passive immunization. A skilled artisan would understand that the substitution of one LPS with another functionally equivalent LPS, lacking O-specific side chain(s) and carrying cross-protective epitope(s), would result in a composition exerting similar, if not better, results/effects. The unique presence itself of broadly cross-protective epitope(s) on a single, easily obtainable LOS core, would motivate a skilled practitioner to substitute Zollinger's generic *E. coli* LPS with Zeigler's specific J5 LOS to produce the instant composition and vaccine for the expected benefit of economically and advantageously immunizing a subject against heterologous Gram negative bacterial sepsis with a single, all-in-one composition, with a

reasonable expectation of success in using such a composition in active or passive immunizations against Gram negative bacterial sepsis. Absent convincing evidence to the contrary, the claimed invention is

With the state of the art teaching that: a) Purified *E. coli* J5 LPS alone induces significantly high levels of antibodies (for example, see Moore *et al.*); b) The detoxified J5 LPS on formulation with a protein retains its antigenicity and immunogenicity, and induces antibodies cross-reactive with multiple heterologous Gram negative bacterial pathogens including *Klebsiella* (Tomita) and, c) Antiserum or purified IgG to J5 LPS shows *in vitro* cross-reactivity with and *in vivo* cross-protection against heterologous Gram negative bacterial pathogens (Dunn *et al.*), the instant invention is *prima facie* obvious over the prior art of record.

New Rejections

7) Applicants are asked to note the new rejections made in this Office Action. Applicants' amendment (submission of new claims) necessitated the new grounds of rejection presented in this Office Action.

Double Patenting

8) A rejection based on double patenting of the "same invention" type finds its support in the language of 35 U.S.C. § 101 which states that "whoever invents or discovers any new and useful process ..... may obtain **a** patent therefor ...." (Emphasis added). Thus, the ter, "same invention", in this context, means an invention drawn to identical subject matter. See *Miller v. Eagle Mfg. Co.*, 151 U.S. 186 (1894); *In re Ockert*, 2456 F. 2d 467, 114 USPQ 330 (CCPA 1957); and *In re Vogel*, 422 F. 2d 438, 164 USPQ 619 (CCPA 1970).

A statutory type (35 U.S.C. 101) double patenting rejection can be overcome by canceling or amending the conflicting claims so they are no longer coextensive in scope. The filing of a terminal disclaimer cannot overcome a double patenting rejection based upon 35 U.S.C. 101.

Claim 20 is provisionally rejected under 35 U.S.C. § 101 as claiming the same invention as that of claim 18 of copending application, SN 08/467,047. The scope of the claimed inventions is identical. This is a provisional double patenting rejection since the conflicting claims have not in fact been patented.

Claim 20 of this application conflict with claim 18 of the copending application, SN 08/467,047. 37 C.F.R 1.78(b) provides that when two or more applications filed by the same Applicant(s) contain conflicting claims, elimination of such claims from all but one application may be required in the absence of good and sufficient reason for their retention during pendency in more than one application. Applicants are required to either cancel the conflicting claims from all but one application or maintain a clear line of demarcation between the applications. See M.P.E.P. § 822.

Claims Rejections - 35 U.S.C § 112, Second Paragraph

9) Claims 19 ad 20 are rejected under as 35 U.S.C §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

(a) Claim 19 is incorrect or incomplete in the recitation "against infection heterologous ....bacteria" (see lines 2 and 3). Amendment to the claim is required. It is suggested that Applicants insert either --by-- or --with-- in between the recitations "infection" and "heterologous".

(b) In claim 20, it suggested that Applicants replace the recitation "a vaccine according to claim 1" with --the vaccine according to claim-- for proper antecedent basis.

Claims Rejections - 35 U.S.C § 112, First Paragraph

10) Claim 19 is rejected under are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for a method of immunizing or treating a second subject using a serum or plasma or specific polyclonal antibody, does not reasonably provide enablement for such a method conferring "protection" against infection by heterologous Gram-negative bacteria or endotoxin-mediated pathology. The specification does not enable any person skilled in the art to which it pertains, with which it is most nearly connected, to make and/or use the invention commensurate in scope with the claim.

The data provided with Dr. Cross's declaration shows that the administration of a vaccine-derived antiserum to animals results in 48% survival of animals against challenge with *Pseudomonas* and 64% survival against challenge with *Klebsiella*. However, 52% and 36% of

immunized or treated animals respectively were not "protected". The full scope of the claims is not commensurate with the scope of the enabling disclosure and undue experimentation would have been required by one of ordinary skill in the art to reproducibly practice the invention as claimed. The enablement (scope) provisions of 35 U.S.C. § 112, first paragraph, are not met and the claim is viewed as non-enabled with respect to its scope.

Rejection under 35 U.S.C. 112, First paragraph

11) Claim 19 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Claim 19 currently includes the limitation "detoxified outer membrane protein" (see line 10). However, there appears to be no support in the instant specification for the added limitation. The specification discloses "purified outer membrane protein derived from *N. meningitidis*", for example, on pages 3, 4 and Example 2), but does not disclose any teachings of producing a "detoxified" OMP. Therefore, this limitation in the claim is considered to be new matter. *In re Rasmussen*, 650 F2d 1212 (CCPA, 1981). New matter includes not only the addition of wholly unsupported subject matter but also, adding specific percentages or compounds after a broader original disclosure, or even omission of a step from a method. See M.P.E.P. 608.04 to 608.04(c).

Applicants are respectfully requested to point to the descriptive support in the specification as filed, for the newly added limitation, or to remove the new matter from the claim.

Claims Rejections - 35 U.S.C § 103

12) New claims 19 and 20 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Ziegler *et al.* (*J. Immunol.* 111: 433-438, 1973) (Ziegler 1973) in view of Zollinger *et al.* (US 4,707,543) and Ziegler *et al.* (*New Eng. J. Med.* 307(20): 1225-1230, 1982) (Ziegler 1982).

Ziegler *et al.* (1973) teach a specific polyclonal antiserum obtained from a rabbit (i.e. the first subject) immunized with an *E. coli* J5 cellular vaccine and a method of passively conferring upon mice (i.e. the second subject) protection against bacteremia by *Klebsiella pneumoniae* (i.e., infection with a heterologous Gram negative bacterium) using the antiserum collected from the first subject (see abstract and page 434). Ziegler *et al.* (1973) do not teach an antiserum

produced using a vaccine comprising a non-covalent complex of purified, detoxified J5 LPS and purified group B meningococcal OMP, and a method of passive protection against endotoxin-mediated pathology using such an antiserum.

The teachings of Zollinger *et al.* as modified by Ziegler *et al.* (Ziegler 1982) are described above.

It would have been *prima facie* obvious to one skilled in the art at the time the invention was made to use Zollinger's LPS-OMP non-covalent vaccine complex as modified with Ziegler's (1982) J5 LPS in Ziegler's (1973) method of producing the antiserum by active immunization of the first subject and use the resultant antiserum in Ziegler's method of passive protection against infection by a heterologous Gram negative bacterium to produce the instant invention with a reasonable expectation of success. A skilled artisan would be motivated to use Zollinger's LPS-OMP non-covalent vaccine complex as modified with Ziegler's (1982) J5 LPS in Ziegler's (1973) method for the expected benefit of avoiding the use of a cellular Gram negative bacterial vaccine, which is known in the art of vaccines to induce undesired reactogencities.

Claims 19 and 20 are *prima facie* obvious over the prior art of record.

State of the Art

13) This section is provided to reflect the state of the art at the time of the instant invention and to address some of Applicants' arguments.

- Bodmer *et al.* (WO 91/01755) teach survival of neutropenic rats immunized with rabbit "polyclonal antiserum against the core glycolipid of LPS of the J5 mutant of *E. coli*" against *Pseudomonas aeruginosa* (see Figure 2). Bodmer *et al.* teach the art-recognized desirability for administration of a cross-protective anti-LPS antibody in order to give broad protection in a number of clinical infections (see page 13).

- Salles *et al.* (*J. Infect. Dis.* 159: 641-647, 1989) teach monoclonal antibodies derived from mice immunized with *E. coli* J5 LPS and LPS-associated proteins. Seven monoclonal antibodies cross-reacted with the LPS of *E. coli* O111, O55, O127 and O128. One of the monoclonal antibodies, B7B3, reacted with the LPS of *Serratia marcescens* and ***Klebsiella pneumoniae***. The D6B4 monoclonal antibody is protective in a lethal endotoxemia model

induced by LPS from heterologous *E. coli* serotypes O111 and O127. The antibodies D6B3 and D4B5 are protective against heterologous infection induced by heterologous *E. coli* O2:K1 serotype (see abstract).

- Moore *et al.* (*Transplantation* 44: 249-253, 1987) teach that endotoxin (ET) has a role in the pathogenesis of graft-versus-host disease (GVDH) and teach the benefits of active and passive immunizations to modulate the severity of GVDH (i.e. an endotoxin mediated pathology) using purified J5 LPS. Moore *et al.* state (see page 252):

> *E. coli* J5 is a rough mutant strain in which CGL (core glycolipid) is exposed on the bacterial cell surface. CGL is largely responsible for the biological properties of ET (endotoxin) and has essentially the same structure in all Enterobacteriaceae (*28*). Braude and others have shown that antisera to *E. coli* J5 protects against a wide range of biological properties of ET (*29*).
> .....naturally occurring anti-*E. coli* J5 antibody protects against Gram-negative shock (*37*), and passive immunization with human anti-*E. coli* J5 antiserum (*38*), high-titer anti-ET plasma (*39*), or human monoclonal anti-*E. coli* J5 antibody (*40*) can protect against the effects of bacteremia and endotoxemia.

- Fenwick *et al.* (*Am. J. Vet. Res.* 47: 1898-1891, 1986) teach the benefit of increased immunity to cross-reacting LPS core antigens of Gram negative bacteria induced by vaccination with the Rc mutant of *E. coli* O111:B4 (strain J5). Compared to the control animals, pigs vaccinated with *E. coli* J5 had lowered mortality with *H. pleuropneumoniae* infection (see abstract).

- Fenwick *et al.* (*Infect. Immun.* 53: 298-304, 1986) teach the mechanism of protection provided by increased immunity to *E. coli* J5 during Gram negative bacterial infections. It is taught that "antibodies against common subsurface components of Gram negative bacterial cell walls correlate with protection from an otherwise lethal challenge with *H. pleuropneumoniae*" (see abstract).

- Young *et al.* (US 4,918,163) disclose endotoxin neutralizing antibodies reactive with Gram negative bacterial endotoxin core exhibiting broad cross-reactivity with Gram negative bacteria of different genera (see abstract). *In vivo* efficacy experiments performed with XMMEN-J5D monoclonal antibodies indicated their protective capacity against *Ps. aeruginosa* and *E. coli* infections (see Table 12).

Nelles *et al.* (*Infect. Immun.* 46: 677-681, 1984) teach one or more antibodies induced by and reactive with *E. coli* J5 LPS, which "exhibit extensive serological cross-reactivity

with a variety of gram-negative bacteria" (see abstract) including *Klebsiella pneumoniae* and *Ps. aeruginosa* (see Table 6).

- Kanegasaki *et al.* (*In: Bacterial Endotoxin: Chemical, biological and clinical aspects.* (Ed) Homma *et al.* Verlag Chemie, pp. 149-158, 1984) teach *E. coli* R (K12) LPS, or *S. minnesota* LPS (lacking O-side chains) complexed with a Gram negative bacterial outer membrane protein and their biological activities. It is known in the art that *E. coli* K12 LPS lacks O-side chains.

- Nelson *et al.* (WO 87/07148) teach a vaccine composition for injection into animals against a Gram negative bacterial pathogen comprising effective dose of a Gram negative bacterial lipopolysaccharide devoid of O-carbohydrate side-chains, exemplified by *E. coli* J5 (see abstract, page 1).

- Tomita (*Dissertation Abstracts International*, vol. 56, 1994, abstract) teaches a protective vaccine comprising **detoxified** *E. coli* J5 bound (covalently) to a protein. On active immunization of mammals (cows), the conjugate vaccine elicit J5 LPS-specific IgM and IgG antibodies, which cross-react with heterologous *Klebsiella pneumoniae*, *Enterobacter* and *Serratia marcescens*. Thus, the detoxified LPS has been shown to contain cross-reactive epitope(s) that are reactive with multiple heterologous Gram negative bacterial pathogens including *Klebsiella*. The art thus shows that the process of detoxification followed by formulation with another protein does not adversely affect the cross-reactive epitopes or the immunogenicity of the J5 LPS.

- Irrespective of whether or not a Gram negative bacterial LPS contains O-side chains, the detoxification of an LPS by treatment with alkali to reduce or eliminate lipid A-associated toxicity does not affect the antigenicity or immunogenicity adversely. For example, Seid *et al.* (*J. Biol. Chem.* 256: 7305-7310, 1981, abstract) teach the preparation of a detoxified Gram negative bacterial LPS-protein conjugate and thus show that detoxification or deacylation of LPS before formulating with a bacterial protein does not affect the antigenic determinants and the immunogenicity of the LPS (see abstract).

Gu *et al.* (*J. Clin. Microbiol.* 30: 2047-2053, 1992) use purified LOS (i.e., LPS

lacking O-specific side chain) alone, without any adjuvant, of a Gram negative bacterial pathogen which also causes sepsis (meningococcaemia), as an immunogen to raise core-specific antibodies, which reacted with multiple serogroups of *Neisseria meningitidis* (see pages 2047 and 2052).

- Tomita *et al.* (*J. Dairy Sci.* 78: 2178-2185, 1995) teach that the disadvantages of *E. coli* J5 LPS such as endotoxicity and poor immunogenicity which limit its use as an immunogen can be overcome by detoxification by alkaline hydrolysis of ester-linked lipid A fatty acids and coupling the detoxified J5 LPS to a protein to elicit a "more defined immune response to common core antigens of coliform mastitis pathogens". The detoxified J5 LPS-protein conjugate is used for active immunization of cows against mastitis (an endotoxin-mediated pathology). The antigenicity and immunogenicity of *E. coli* J5 LPS was not altered by detoxification and conjugation. Immunization of cows with the conjugate vaccine elicited a significant IgG and IgM immune responses to J5 LPS and J5 whole-cell antigens. Tomita *et al.* teach that active immunization against coliform bacteria is a method to control coliform mastitis and that core-specific antibody titer has to be increased to decrease further the incidence of clinical cases and the severity of coliform mastitis. Tomita *et al.* teach that the J5 LPS has the advantage of having a common antigenic structure among coliform pathogens. Tomita *et al.* teach that increased IgG antibody titer to J5 is associated with reduced rate and severity of clinical coliform mastitis. Tomita *et al.* discuss the studies by Ziegler *et al.* (*J. Immunol.* 111: 433-438, 1973). Tomita *et al.* state that laboratory animals immunized with J5 bacterin produced core-specific antibodies that were cross-reactive and protective against heterologous coliform infection and that protection was attributed to elevated antibody titer (see entire document).

- Tomita *et al.* (*J. Dairy Sci.* 78: 2745-2752, 1995) teach the isolation of serum IgG from cows immunized with an *E. coli* J5 LPS conjugate vaccine and show that it is highly cross-reactive with the LPS of *E. coli* J5, *E. coli* O111:B4, *Serratia marcescens*, *Klebsiella pneumoniae* and *Salmonella typhimurium* (see abstract).

- Dale *et al.* (*J. Infect. Dis.* 166: 316-325, 1992) teach that human vaccination with *E. coli* J5 mutant induces cross-reactive bactericidal antibody against *Neisseria gonorrhoeae* LPS.

McCabe *et al.* (*Prog. Clin. Biol. Res.* 47: 107-117, 1980) teach the potential use of shared antigens for immunization against Gram-negative bacillary infections by active and

passive immunization using rough mutants.

Braude *et al.* (*J. Infect. Dis.* 136: S167-S173, 1977) teach both active and passive immunization against bacteremia and endotoxemia due to *Ps. aeruginosa* using *E. coli* J5.

- Cryz *et al.* (*Eur. J. Clin. Microbiol.* 4: 180-185, 1985, abstract) show that active immunization with O-polysaccharide-deficient lipopolysaccharides derived from *E. coli* J5 afforded substantial protection against a strain of *Pseudomonas aeruginosa.*

- Lugowski (*Acta Biochim. Pol.* 42: 19-24, 1995) teach:

> Endotoxins are responsible for initiation of septic shock which increases the number of fatalities in Gram-negative bacteremia among hospital patients. The mortality from septic shock is still high despite recent developments in anti biotic therapy. These substances are unable to decrease the level of free lipopolysaccharides in the bloodstream. Another approach to the treatment and prevention of septicaemia involves simulation of an immune response against LPS. It was found that immunization with core structures of endotoxin conjugated with proteins protected animals against infections and endotoxin shock. Anticonjugate sera are of great interest because they are directed against common parts of LPS and therefore could have cross-protective potencies towards many gram-negative rods.

- Davis *et al.* (*J. Exp. Med.* 147: 1007-1017, 1978, abstract) teach the superiority of J5 core glycolipid antibodies, both purified IgG and antiserum, in neutralizing meningococcal endotoxin and its role in interrupting the devastating course of meningococcal endotoxemia regardless of the capsular serogroup of the infecting meningococci.

Remarks

14) Claims 1-3, 5-8, 15-17, 19 and 20 stand rejected.

15) **THIS ACTION IS MADE FINAL.** Applicants are reminded of the extension of time policy as set forth in 37 C.F.R 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 C.F.R 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the mailing date of this final action.

16) Papers related to this application may be submitted to Group 1600, AU 1641 by facsimile

transmission. Papers should be transmitted via the PTO Fax Center located in Crystal Mall 1 (CM1). The transmission of such papers by facsimile must conform with the notice published in the Official Gazette, 1096 OG 30, November 15, 1989. The CM1 facsimile center's telephone number is (703) 308-4242.

17) Any inquiry concerning this communication or earlier communications from the Examiner should be directed to S. Devi whose telephone number is (703) 308-9347. The Examiner can normally be reached on Monday to Friday from 8.00 a.m. to 4.00 p.m. A message may be left on the Examiner's voice mail system.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, James Housel, can be reached on (703) 308-4027.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group receptionist whose telephone number is (703) 308-0196.

August 1999

James C. Housel
JAMES C. HOUSEL 8/16/99
SUPERVISORY PATENT EXAMINER